escape into the auditory canal. Should this occur, the injection must be repeated two or three times until the covering of the superior wall bulges downward, showing that sufficient fluid is retained.

The syringe is cautiously withdrawn and a small pledget of cotton saturated with tonogen is pressed against the field of operation. After waiting from ten to fifteen minutes to allow the anesthetic to take effect, the operation can be performed without the slightest inconvenience to the patient.

On the completion of the operation, the field is dusted with a mixture of equal parts of anesthesin and boracic acid to counteract the severe pain which would otherwise develop in an hour or two following the operation. This indication is fully met by this powder, as in the cases where it has been used the patients have not complained of any pain subsequent to the operation.

The syringe used for these injections is of metal, holding 1 c.c.; and with a needle modified by Neumann. A pair of half rings (which are detachable) have been added to the syringe to facilitate the injection.

In conclusion, we wish to express our gratitude to Professor Politzer and his assistants for the exceptional opportunities afforded us for study during our term of service in the clinic at Vienna.

## PUS TUBES IN THE MALE, AND THEIR SURGICAL TREATMENT.

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Due attention is not generally accorded to the fact that the genital duct proper in the male—seminal vesicle, vas deferens and epididymis—is just as prone to suppuration as is the urethra. Suppuration in the vesicle, it is true, has been recognized, in recent years, as a frequent feature of gonorrhea; but the extension of this infection to the epididymis—frequent and familiar though it be—is not generally considered a suppurating process. Even the best of our modern treatises expressly state that suppuration is exceptional in epididymitis, gonorrheal or other; they consistently prescribe the usual medical treatment, but do not advise the evacuation of pus, except in those rare cases in which fluctuation becomes distinct.

The pus infections of the epididymis and of the vas deferens present a complete though neglected analogy with the familiar pus infections of the fallopian tubes. An epididymitis following prostatic pus infection, whether gonorrheal or other, is usually suppurative; pus tubes are quite as common in the male as in the female; hence the rational treatment of such acute epididymitis should frequently be surgical, i. e., it should include the evacuation of pus by incision.

In the mildest cases such incision may be needlessly radical, since the pus may presently find the natural channel into the urethra; on the other hand, the acute hydrocele and the scrotal edema, which so often accompany severe acute epididymitis, are infallible signs of suppuration in the epididymis, and the incision is followed by relief from pain and subsidence of the swelling. Even when no pus escapes, the relief of tension affords marked comfort and insures a relatively speedy convalescence.

If direct exit for the pus by incision into the epididymis be not provided, the gathering pus often burrows along the vas into the urethra; and thus is explained the familiar phenomenon that decrease of the pain of epididymitis is accompanied by marked increase in the discharge from the meatus. Moreover, persistent suppuration in the epididymis may feed the gleet which follows, just as pus-tubes in the female feed a leucorrhea; and this gleet may recur indefinitely, in spite of elaborate urethral and internal medication, until the hard and tender swelling of the epididymis is incised and the contained pus evacuated.

In other words, the successful treatment of a gleet sometimes requires incision into the epididymis.

Again, chronic epididymitis through pus infection may require surgical treatment; persistent pain and tenderness following the acute infection (sometimes miscalled neuralgia of the testis) is relieved by incision or excision of the epididymal pus focus; destruction of the testis by extension of the pus infection from the epididymis may be prevented by the same treatment.

The lower end or tail of the epididymis presents the chief swelling in acute or chronic epididymitis (pus infection), since in this tail is coiled the greater portion of the 20 feet of the epididymal tube; and into this lower end the incision usually should be made. Simple puncture with bistoury or aspirating needle may sometimes suffice; yet drainage is better assured by an incision a half inch or more long, the edges of the pus cavity being loosely stitched to the cut edges of the skin, and a small drain inserted.

In a few cases I have practiced a therapeutic measure which, according to my present information, is novel, namely, injections into the vas deferens and seminal vesicle, through a needle introduced into the vas just above the epididymis. As the dilated upper extremity of the vas, the ampulla, bears about the same relation to the vesicle that the auricle does to the ventricle of the heart, a liquid injected into the vas easily reaches the vesicle; this can be clearly demonstrated by injecting Prussian blue in water into the vas of the fresh subject.

The therapeutic value of such injection is not yet accurately determined; at present it can be merely affirmed that this is a practicable way—and as yet the only way—for directly medicating the male genital tube, including the vesicle, which is so commonly and so persistently infected by various pus bacteria and by the gonococcus.

# *Special Article*

## IMMUNITY.

### CHAPTER XIII.

### THE BACTERICIDAL PROPERTY OF SERUMS.

**The Cellular and Humoral Theories.**

**There are two theories of note which concern the ability of the body to destroy bacteria. One of these is the phagocytic theory of Metchnikoff, according to which micro-organisms can not be destroyed without the direct or indirect participation of the phagocytic cells; this is the cellular theory. The second, the humoral theory, supposes that the power resides in the fluids of the body alone; in its improved form it now holds that antibacterial immunity should be considered cellulo-humoral, and that whatever bactericidal power the body fluids have is derived primarily from the body cells. Many who appreciate the action of the serum, however, also believe that phagocytic cells may take up and, in certain instances, destroy micro-organisms; this combined view seems best to fit the facts as known at present.**

Antibacterial, bactericidal and bacteriolytic are three terms which are used in a rather loose, interchangeable way, although they are not strictly synonymous. A bactericidal serum is one which is able to kill bacteria, as the term implies; if at the same time it dissolves the organisms it is bacteriolytic. Inasmuch as some serums do kill bacteria without dissolving them (typhoid), while others have the dissolving power (cholera), the distinction is one of significance. In either case the serum is, of course, antibacterial. For lack of a more concise English term, bacteriolysis is used to designate the process in which bacteria are killed by serums regardless of the dissolving power of the latter. Bacteriolysin refers to the substances of the serum which accomplish this action. The means of determining the bactericidal power of a serum was indicated in a previous chapter. Bacteriolysis is best observed with the organism of cholera and its antiserum as described later under the title of the Pfeiffer experiment.

**Bacteriolysis and Bacteriolysin.**

Bacteriolysins are far more complex than antitoxins, agglutinins and precipitins. One may best appreciate the present situation by briefly tracing their history as they were evolved from the relatively simple alexins of Buchner.

Following the investigations of Fodor, Behring and others, which showed that normal blood may kill bacteria in the test-tube, and after additional facts were obtained by Nuttall, Buchner demonstrated that it was not necessary to use the full blood in order to obtain the bactericidal action, but that the serum alone had a similar effect. He spoke of the antibacterial substances collectively as alexins (substances which ward off), taking the reasonable view that natural immunity to bacteria depends on their presence.

**Alexins.**

Alexins were very sensitive substances; they disappeared spontaneously from serums in a few days, were destroyed by a rather low degree of heat (55° C.), by acids and alkalies, and were active only in the presence of certain salts, especially sodium chlorid. A striking feature of alexins, as distinguished from chemical bactericides, was their marked selective action on bacteria. The alexins of animal A might destroy one micro-organism readily and affect another little or none at all, whereas those of animal B might have opposite selective characteristics. The increased bactericidal power of serum which develops during immunization or infection, goes hand in hand with the increased resistance of the individual against the infection. The alexins have undergone a specific increase; they are now immune alexins or, as we say to-day, immune bacteriolysins. The new alexins are identical in their properties with those which occur normally; they exist merely in greater concentration in the acquired immunity.

**Selective Action.**

Work which was instituted by Pfeiffer and developed further by others led the way to a more correct understanding of the nature of alexins. Pfeiffer studied the bactericidal action of serums in the body of the living animal, i. e., in the peritoneal cavity. His most classic results were obtained with the organism of cholera. Guinea-pigs are immunized against this microbe by injections of the killed or living organisms. We have already learned of this process as that of active antibacterial immunization. When the animal is well immunized the experiment is begun by the injection of a quantity of culture which would be fatal to an unimmunized animal. At intervals during the next twenty or thirty minutes small amounts of the peritoneal fluid are removed for microscopic examination. In order to obtain the fluid, fine pipettes are drawn out in the flame, an incision is made in the skin, and the abdominal wall is then punctured with the pipette; the fluid flows into the tube by capillary attraction. A portion may then be examined in a hanging-drop preparation or dried on a cover-glass, fixed in the flame and stained with a dilute solution of carbol-fuchsin. In the hanging-drop preparations it is first noticed that the organisms have lost their motility; the comma- and S-shaped forms soon become spherical and at first appear swollen and clear, whereas in later preparations they gradually decrease in size and show a very rapid vibrating movement, the so-called Brownian movement, which is purely physical in nature. In the course of from twenty to thirty minutes the organisms have been completely dissolved. These changes may also be followed in the stained specimens, in which the altered cells eventually appear as fine red granules.

**The Phenomenon of Pfeiffer.**

As Metchnikoff, Bordet and others have shown, the same phenomenon may be followed without the intervention of the animal body, by mixing perfectly fresh anti-cholera serum with the vibrio and mounting as a hanging-drop preparation. The slide must be kept at the temperature of the body by means of a warm stage. The reaction, however, is far less vigorous than when it takes place in the peritoneal cavity and the solution of the cells may not be complete. No bacterium is so completely dissolved under these conditions as the vibrio of cholera, although the typhoid bacillus and similar organisms undergo some changes in their form.

**The Experiment in Vitro.**

The experiment of Pfeiffer may also be conducted in the abdominal cavity of a non-immune guinea-pig, if anticholera serum is injected in conjunction with the organism (passive antibacterial immunization). This is the classic Pfeiffer experiment. The immune serum should be of such strength and should be given in such quantity that the animal is saved in spite of the ten fatal doses of culture which the typical experiment demands. Experiments brought to light a condition which seemed paradoxic; an old immune serum which had lost its bactericidal power as manifested *in vitro*, or one in which the alexins had been destroyed by a temperature of 60° C., showed its original protective power when the experiment was performed in the peritoneal cavity. Furthermore, when an inactive immune serum was injected into the cavity, allowed to remain for a time and then withdrawn, its bactericidal power for experiments *in vitro* was found to be re-established. On the basis of these facts, Pfeiffer concluded that the specific substance is present in the immune serum in an inactive form, and that it becomes active as a result of contact with living tissue cells, supposedly the endothelial cells of the peritoneum. According to this conclusion, an inactive serum could become active again only after its introduction into the body.

**The Activation of an Inactive Serum by the Tissues.**

It remained for Bordet to show, on the contrary, that the serum could again be made active for experiments in the test-tube without the aid of living cells. This he accomplished merely by adding to the heated immune serum a small amount of fresh normal serum from the guinea-pig or goat, the quantity of normal serum which was used not being in itself bactericidal. We have, then, two serums which when combined are bactericidal, but when separated are inactive. The destruction of the active property of a serum by heat or by other means is called inactivation, and the re-establishment of its power by the addition of fresh normal serum is reactivation. The immune serum, when heated to 55 to 60° C., loses something which is essential to its activity, and this something may be replaced by the normal serum. That the substance in the normal serum is identical with that which was destroyed in the immune serum is indicated by the fact that it is destroyed by the same degree of heat; a heated normal serum will not reactivate an immune serum.

**Inactivation and Reactivation.**

The conclusion of Bordet that the bactericidal power of a serum depends on the combined action of two substances has been substantiated by numerous investigators. The properties of the two substances will be given in the succeeding chapter. At this point it is sufficient to say that these are the substances which in recent years have become familiar under the names of amboceptor and complement and their various synonyms. One of them, the

**Two Substances in a Bactericidal Serum.**

amboceptor, is heat-resistant (thermostabile), i. e., it is not destroyed at 56° C., whereas the other, the complement, is susceptible to heat (thermolabile), being destroyed at that temperature which killed the alexins of Buchner. The term alexin is still applied by some writers to the thermolabile substance (complement), but its original significance has been lost.

**Specificity.**

The specificity which prevails among antitoxins and agglutinins is found also in the action of bactericidal serums. When an anticholera serum is injected into the peritoneal cavity of a guinea-pig, protection is not afforded against other vibrios or other pathogenic organisms. The specificity is so great that the reaction of Pfeiffer may be used for the identification of bacteria. If one has in hand an unknown vibrio, its identity or non-identity as the organism of cholera may be determined by injecting it, in conjunction with anticholera serum, into the peritoneal cavity of a guinea-pig; if the microbe is transformed into granules it is the vibrio of cholera, otherwise it is not. Other bacteria may be identified in a similar manner by the use of the proper serums. In spite of this high specificity the group reaction may occur even with bactericidal serums. An antityphoid serum may have a stronger bactericidal action than a similar normal serum against organisms which are closely related to that of typhoid, as the colon bacillus, but the power is never so strong as against the typhoid organism. Hence, for the purpose of identifying bacteria, the method of serum dilution is as decisive as in connection with agglutination.

**Group Reaction.**

Bactericidal serums are not obtained with equal readiness for all organisms. We are most familiar with those which are yielded by immunization or infection with the microbes of cholera, typhoid, plague, the colon bacillus and related bacteria. Many other bacteria yield neither antitoxins nor bactericidal substances through immunization, nor are the serums protective when injected into animals; the pneumococcus, streptococcus, tubercle bacillus and others. Individuals who have suffered infections from these organisms are endowed with little or no immunity against subsequent attacks. The pneumococcus grows well in the serum of a convalescent from pneumonia. Thus it is seen that the field of antibacterial immunity is filled with many contraindications if one attempts to interpret it merely on the basis of the bactericidal properties of serums as they are manifested *in vitro*.

**The Effect of Bactericidal Serums on Endotoxins.**

In a preceding chapter micro-organisms were divided, first, into those which secrete soluble toxins, immunization with which causes the formation of antitoxins, and, second, those which do not secrete such toxins and for which no manipulations of cultivation and immunization known at the present time are successful in stimulating to the formation of antitoxins. After all, it seems plain that the bacteria of the second group must be pathogenic because of toxic substances which they carry with them into the body. In view of the fact, however, that they do not secrete soluble toxins in culture media, it is held that their toxic properties are integrally associated with the bacterial protoplasm; they are the endotoxins spoken of previously.

The question naturally arises: Does a bactericidal serum in dissolving or killing its homologous organism at the same time neutralize the endotoxin? On the basis of very positive experiments which have been performed, especially by Pfeiffer, it is evident that the serum has no such action. In the experiment of Pfeiffer, one may inject into the abdomen a sufficient quantity of anticholera serum to kill all the organisms which have been introduced, and yet the animal dies with the intoxication of cholera. Furthermore, if one considers a culture of the cholera vibrio, which has been killed by heat, as representing so much cholera toxin, anticholera serum protects against no more of it than does the same quantity of normal serum. It is believed that anticholera and similar immune serums may even increase intoxication by dissolving the bacteria and thus liberating an excess of toxins.

In spite of the classification of bacteria into the two groups just cited there are a few microbes which, according to manipulation, cause the formation of either an antitoxic serum or a bactericidal serum. In general it may be said that the character of the serum depends on the bacterial constituent which is used for immunization. If the diphtheria bacillus itself, or the pyocyaneus bacillus, is injected, the toxin having been washed away, bactericidal serums are formed, whereas if toxins alone are introduced, antitoxins are the result.

**The Bactericidal Power in Relation to Natural Immunity.**

In view of the fact that the increased bactericidal power of the serum of one who has had typhoid fever, cholera or one of the other diseases in this group, is so distinctive and so potent, it is generally believed that the immunity depends on this factor. May we with equal justification conclude that the bactericidal powers of normal serums are correct indices of the natural antibacterial immunity of the animals? Although this relationship has been found to exist in a number of cases, there are other instances in which it does not prevail. Anthrax may be cited as an example; *in vitro* the serum of the rabbit is strongly bactericidal for the bacillus although the animal is exceedingly susceptible; on the other hand, the dog has much more resistance to anthrax although its serum has but slight bactericidal effect on the organism. Experiments of some importance have to do with the ability of bacteria to absorb the homologous bactericidal substance from a serum when the two are mixed in test tubes. Hence, if natural antibacterial immunity depends on the bacteriolysin which is present in the circulation, a large mass of the bacterium when injected intravenously should absorb or fix the bactericidal substances; as a consequence, serum which is drawn later should show a great decrease in its bactericidal power for the organism which was injected. Although results of this nature have been obtained by a number of competent investigators, they are not without exception. In the same connection fatal infections should be accompanied by a decrease of the natural bactericidal power of the serum for the organism involved. This has been found to be true in man in relation to plague, and in some animal infections. At present general statements concerning this point can not be well made.

**Origin of Bactericidal Substances.**

We have little positive knowledge concerning the organs which form the bactericidal substances in acquired immunity. Pfeiffer and Marx, in relation to cholera, and Wassermann in typhoid, found that the spleen and the hemopoietic organs in general contain the immune bodies in greater concentration than the blood serum, and in immunization experiments the bodies may be demonstrated in these organs at a time when they are absent from the circulation. This fact is generally accepted as proof of their formation at these points. Wassermann and others have demonstrated the presence of complement in the leucocytes, and Metchnikoff holds that it is produced only by such cells.

**Standardization.**

The standardization of bactericidal serums is at present more of theoretical than of practical interest, because of their limited therapeutic use. Their values can not be determined with the same degree of accuracy with which one measures the unit of antitoxin. One may deliver from a pipette a definite quantity of toxin and if the toxin has been well preserved the same quantity may be obtained at any subsequent time. It is impossible to preserve a culture of living bacteria so that the number of the organisms and the virulence of the culture remain constant, nor will two cultures made at different times contain the same number of cells in a given volume. Hence, standard cultures which are necessary for the systematic valuation of serums are not easily available. One may use a definite volume of a bouillon culture of an organism which has grown for a certain number of hours, but in all likelihood no two cultures would contain the same number of organisms.

Pfeiffer uses the normal loop which has been mentioned, i. e., one which will take up from a surface of agar two milligrams of the bacterial mass. The culture must have grown for a definite period, eighteen to twenty-four hours. Tests having some value may be made in the test-tube, provided the antibacterial serum is used when perfectly fresh, i. e., when its complement has not yet degenerated; this, of course, gives one only the bactericidal power as it is manifested outside the body, and, as stated, this may not be a correct index of the protective power of the serum when it is injected into the living animal. For the test-tube experiment various dilutions of the serum are made, as 1 to 10, 1 to 100 and 1 to 1,000, and a similar quantity of each dilution is mixed with a given mass of the culture; the mixtures are then placed in the thermostat for a number of hours. At the end of this time plate cultures are made from each of the mixtures, the plates put aside for twenty-four hours, and the colonies which have developed are then counted. The quantity of serum required to kill all the bacteria used may thus be determined.

When the protective power of the serum is determined by animal experiment it is not essential to use the serum when fresh; in fact, the native complement in the immune serum may be disregarded, or, preferably, it may be destroyed by heat. If the latter procedure is adopted, or if an old serum is used in which the complement has degenerated, its reactivation is accomplished through the complement which is present in the body of the experiment animal. It will appear in more detail in the succeeding chapter that a given antiserum requires a particular complement for its reactivation, and that this complement may be present in some animals and absent in others. Hence, it must be known in advance that the animal chosen for the experiment possesses the suitable complement.

It is also necessary to know the virulence of the culture with which the antiserum is to be tested. It is possible to maintain some organisms at a rather constant virulence by passage, i. e., infecting animals with the microbe and recultivating it from the tissues. With others, abundant controls must be made at the time the serum is tested in order to know at that moment the precise virulence of the culture. In all probability it requires more serum to protect against very virulent cultures than against those of less virulence.

To find the value of anticholera serum Pfeiffer prepares dilutions similar to those mentioned above, and to the same quantity of each dilution adds ten fatal doses of a virulent culture of the vibrio of cholera. These are injected into the peritoneal cavities of three guinea-pigs and after periods of forty to sixty minutes hanging-drop preparations are made from the peritoneal fluid of each animal to determine the formation of the characteristic granules; the highest dilution which causes this change in the cells stamps the value of the serum. The animal must at the same time be protected against the ten fatal doses of the culture.

The value of an antityphoid serum may be determined in the same way, the result being judged by the protection which is afforded the animal rather than by the formation of granules.

Antityphoid, antiplague, and some other serums are also tested by injecting the serum twenty-four hours in advance of the culture.

**Non-Specific Increase in Resistance.**

In contrast to the specific immunization which may be accomplished with an immune serum, it is important to recognize that a non-specific increase in resistance may be caused by the injection of a number of substances, which in the test-tube have no destructive action on the bacteria. Issaeff injected into the peritoneal cavity such substances as bouillon, tuberculin and sterile urine, and found the resistance of the animals increased to peritoneal inoculation of virulent organisms. Normal serum has a similar effect, but, in this instance, the bactericidal substances of the serum may be of influence. Supposedly, this non-specific resistance is local, and it appears to depend on the attraction of an increased number of phagocytes and of additional complement to the peritoneal cavity. The suggestion recently made that preceding laparotomy nucleinic acid be injected into the abdominal cavity, in order to increase the local resistance, has its foundation in the experimental work just cited.

*(To be continued.)*

## Clinical Notes

### DIPHTHERIA ANTITOXIN IN THE TREATMENT OF GOITER.

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During the summer of 1903 I had occasion to treat a family with diphtheria; one patient died from the laryngeal form after tracheotomy had been performed and large doses of antitoxin had been administered. An aunt acted as a nurse; she had exophthalmic goiter, with all the classic symptoms. No immunizing dose of antitoxin was given her, and she was infected with moderately severe pharyngeal diphtheria. I immediately injected 1,500 units of antitoxin and repeated the dose on the following day, combined with local antiseptic and internal supportive treatment, with the result that at the end of the week she was convalescent.

Four months later I saw this patient and noticed that the large tumor of the neck had disappeared, and that the eye symptoms were a trifle better. She stated that since the attack of diphtheria the goiter had grown smaller to such an extent that she noticed the muscle lines of the neck. Her pulse rate became normal and the museular twitchings and nervousness ceased. She is the only person I ever heard of who confessed to being glad she had had diphtheria. The case was of eight years' duration. One year has now elapsed with no return of the tumor. Since then I have had two cases of goiter, with the following experiments with diphtheritic antitoxin:

CASE 1.—Miss T., aged 22; simple goiter; no eye symptoms; disease of six years' duration. Her father died of heart disease.

*Examination.*—She was robust, had no anemia, but was very nervous and had palpitation of the heart. Pulse was regular, 90; no heart lesion. Tumor measured 33 cm.

*Treatment.*—July 16, 1904, I injected 2,000 units of antitoxin. July 25, 1904, I repeated the dose.

*Result.*—The pulse dropped to 85, tumor measured 32 cm. and she felt better. August 10, pulse 80, tumor 32 cm.; no palpitation or nervousness. A week later pulse was 72, but there was no apparent reduction of the tumor. I lost track of the case on account of removal of the patient. Four months later a friend of hers informed me that the tumor was nearly gone. She never complained again of her condition, and could button her collar bands easily on account of their looseness.

CASE 2.—Mrs. E., aged 35, had exophthalmic goiter of a pronounced type and of two years' duration, but worse during the past four months. No family history of goiter.

*Examination.*—She complains of being tired and is very nervous, with accentuated muscular twitchings. Pulse soft, 120; she is anemic, has a heart murmur, ankles are swollen and there is albuminuria. Tumor measured 35 cm. and there was a loud bruit.

*Treatment.*—July 18 I administered 2,000 units antitoxin and on July 28 repeated the dose; pulse 82.

*Result.*—August 8, pulse 80, tumor 33.5 cm. She felt stronger, there was no palpitation, the muscular twitchings were controlled and heart symptoms improved. At this visit I asked her to purchase more antitoxin, but she could not, on account of the expense. Five months later she stated that she felt perfectly well and that she was able to do her housework and to perform other duties which she had been unable to perform before treatment. On examination I found the tumor very much decreased, over half the parenchyma having disappeared, leaving a bunch of pulsating vessels that felt like a